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(54) Title: USE OF NONIMMUNOSUPPRESSIVE CYCLOSPORIN DERIVATIVES FOR HAIR GROWTH

(57) Abstract: The present invention relates to agents for treating alopecia and stimulating hair growth comprising an active ingredient of nonimmunosuppressive [γ-hydroxy-N-methyl-L-leucine[4]] cyclosporin B, C, D, G having excellent hair growth-promoting effect, wherein the hydroxyl group is added to the γ carbon position of No. 4 N-methyl-L-leucine of cyclosporin B, C, D, G by the microorganism.

USE OF NONIMMUNOSUPPRESSIVE CYCLOSPORIN DERIVATIVES FOR HAIR GROWTH

Technical Field

The present invention relates to a hair growth promoter comprising a cyclosporin derivative as an active ingredient which has much low level of immunosuppression and maintains a good hair growth.

Background Art

Opinions of poor blood circulation, excessive male sex hormone functioning, seborrhea, scalp function deterioration by peroxides, bacteria, etc., hereditary factors, aging stresses, etc have been argued as reasons for the hair loss. However, explicit reasons for hair loss have not been identified up to now, recent trends are that population worrying about hair loss caused by stress increase due to dietary habit change, social environment, etc. is being increased, its age is also being lowered, and female hair loss population is also being increased.

A preparation containing minoxidil which is most widely used until now in the treatment or prevention of this alopecia is one of two hair growth stimulating ingredients which have received a permission of the U.S. Food and Drug Administration. Minoxidil has become a medication that is now more famous as a hair hair growth stimulating compound since trichogenous (i.e., hair growth stimulating) phenomena occur due to side effects in the application although minoxidil was a high blood pressure treating agent that had been originally developed for the purpose of blood pressure drop.

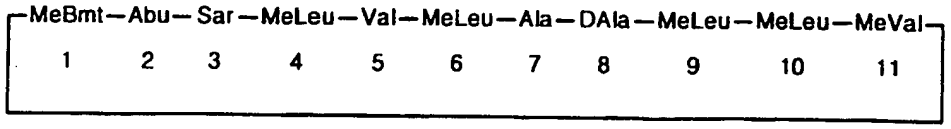
Additionally, the Merck Corporation's recently commercially available Propecia, principal ingredient being finasteride, inhibits the transformation of male

sex hormone testosterone into dehydrotestosterone, a more potent male sex hormone.

Although the finasteride, which is now being commercially available after 1 mg tablet was received usage permission from Food and Drug Administration in December 1997, showed notable effects as a result of clinical tests partial side effects of male sex function suppression have also been reported (J. Am. Acad. Dermatol., 1998; 39; 578 ~ 589). However, searches and studies on superior hair growth promoter are actively being pursued since the medication like minoxidil does not have excellent clinical effects either and due to concern over side effects.

It has been reported that cyclosporin is not only a representative immunosuppressant, but it also brings about various physiological effects such as nephrotoxicity, hepatotoxicity, high blood pressure, hair growth-stimulating effect, gingival over growth, and antimicrobial effects against for viruses, fungi, and protozoa (Advances in Pharmacol., 1996; 35; 114 ~ 246 and Drug Safety, 1994; 10 310 ~ 317). A representive cyclosporin A is shown in the following Structural Formula 1 as a cyclic peptide with 11 amino acids comprising various N-methyl amino acids, and D-alanine at the No. 8 position.

[Structural Formula 1]



where MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine; Abu is L-α aminobutyric acid; Sar is Sarcosine; MeLeu is N-methyl-L-leucine; Val is L-valine; Ala is L-alanine; DAla is D-alanine; and MeVal is N-methyl-L-Valine.

Furthermore, the amino acid form of the above cyclosporin A is L-configuration if there is not any mention. As shown in Structural Formula 1, a residue number of amino acid is assigned 1 for MeBmt and clockwisely, 11 for MeVal (N-methyl-L-valine). Nomenculature of various derivatives of cyclosporins A to Z follows methods commonly used (Helv. Chim. Acta, 1987;70;13-36). For example, cyclosporin molecules, in which only L-α aminobutyric acid is substituted with L-alanine, L-threonine, L-valine or L-norvaline, are expressed by cyclosporin B, C, D or G, respectively. The name of the derivative, in which hydroxyl group is substituted in γ carbon of N-methyl-L-leucine at no. 4 of cyclosporin by microorganism procedure, is expressed by [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin, describing only the substituted residue. For example, when the hydroxyl group is substituted in γ carbon of N-methyl-L-leucine at no. 4 of cyclosporin B, C, D, or G, by microorganism procedure, [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin B, [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin C, [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin D, and [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin G are assigned, respectively. Furthermore, the term "cyclosporin derivative(s)" as used herein means a derivative substituted with γ-hydroxy-N-methyl-L-leucine, in which hydroxyl group is attached to γ carbon of N-methyl-L-leucine, no. 4 residue of a cyclosporin molecule by the metabolism of microorganisms. For nomenclature of the N-methylated amino acid, as used herein, for example, MeLeu means N-methyl-L-leucine and MeVal means N-methyl-L-valine.

Possibilities for the development of cyclosporin as a new hair growth stimulator using excessive hair growth side effects have been reviewed in a variety of studies. Among them, animal hair growth-stimulating tests (Arch, Dermatol. Res., 1996; 288; 408 ~ 410),

human alopecia areata (J. Am. Acad. Dermatol., 1990; 22; 242 ~ 250), human male pattern alopecia (J. Am. Acad. Dermatol., 1990; 22; 251 ~ 253 and Skin Pharmacol., 1994; 7; 101 ~ 104), and protection from chemotherapy-induced alopecia (Clin. Lab. Invest., 1995; 190; 192 ~ 196 and J. Pathol., 1997; 150; 1433 ~ 1441) have been reported and have shown about 100 times superior effects than minoxidil when compared to as the results of mouse backside test. Various patents have been applied as the results of efforts to utilize cyclosporin as a treatment for male pattern alopecia based on these results.

For example, hair-growth promoters using these cyclosporin and derivatives in Japanese Laid-open Patent Publication Nos. Showa 60-243008, Showa 62-19512, and Showa 62-19513, cyclosporin derivatives with No. 8 position changed (European Laid-open Patent Publication No. 0414632B1), and isocyclosporin (World Laid-open Patent Publication No. 93/17039), etc., are provided and hair-growth stimulators in which transdermal absorption of cyclosporin is superior are provided in U.S. Patent No. 5,807,820 and U.K. Patent No. 2,218,334A. However, there are many limits in the application due to the severe side effects of immunosuppression although all cyclosporin groups used here have superior trichogenous effects in general alopecia.

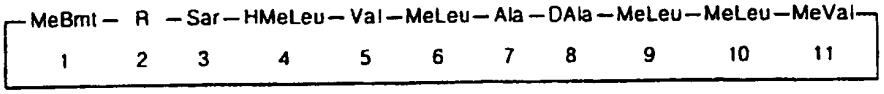
Disclosure of the Invention

Therefore, by taking into account of problems of the above conventional technologies and perceiving the discovery that trichogenous effects of cyclosporin do not necessarily mean the prerequisite for degree of immunosuppression of cyclosporin molecules (Iwabuchi et al., J. Dermatol. Sci., 1995; 9; 64 ~ 69), it is an object of the present invention to provide a new hair growth promoter by employing various molecular transformation of cyclosporin.

It is a further object of the present invention to provide a new hair growth promoter comprising cyclosporin derivatives as an active ingredient, in which immunosuppression is lessened comparing that of non-transformed cyclosporin while superior trichogenous effects are maintained.

In order to accomplish the above objects, the present invention provides a hair growth promoter comprising an active ingredient of cyclosporin derivatives represented as in the following Chemical Formula 1 in which hydroxyl group is added to γ carbon position of No. 4 N-methyl-L-leucine of cyclosporin by the microorganism metabolism procedure.

[Chemical Formula 1]



where R is Ala, Thr, Val or Nva, in which Ala is L-alanine, Thr is L-threonine, Val is L-valine, Nva is L-norvaline; MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine; Sar is Sarcosine; HMeLeu is γ-hydroxy-N-methyl-L-leucine; MeLeu is N-methyl-L-leucine; DAla is D-alanine; and MeVal is N-methyl-L-Valine.

Furthermore, the present invention provides a hair growth promoter, wherein the composition is prepared in a form of liquid formulation, spray, gel, paste, emulsion, cream, conditioner, or shampoo.

Brief Description of the Drawings

The above objects, and other features and advantages of the present invention will become more apparent after a reading of the following detailed description when taken in conjunction with the drawings, in which:

FIG. 1 is the high pressure liquid chromatography results for [γ-hydroxy-N-methyl-L-leucine^4] cyclosporin C transformed by microorganisms (retention time : 19.979 minute) and cyclosporin C that is not transformed by microorganisms (retention time : 23.132); and

FIG. 2 is the high pressure liquid chromatography results obtained by re-injecting [γ -hydroxy-N-methyl-L-leucine^4] cyclosporin C after collecting.

Best Mode for Carrying out the Invention

The present invention is described in detail as following:

The inventors have studied to discover a cyclosporin derivative maintaining trichogenous effects without immunosuppression in order to develop a new trichogenous ingredient. We subjected *Tolypocladium inflatum* ATCC 34921 as a parent strain for cyclosporin production to UV irradiation to create a mutant strain. The cyclosporins obtained from the mutants were transformed by using microorganisms. In the hair growth evaluation tests, the present cyclosporin derivatives have trichogenous effects comparable to cyclosporin A while their immunosuppressions are considerably reduced.

The present invention will be understood in detail with referring to the following examples, which is shown and described, simply by way of illustration of the present invention not of restriction to the scope of the invention.

EXAMPLES

EXAMPLE 1

This example describes a mutation of *Tolypocladium inflatum* ATCC 34921 and a preparation of a group of cyclosporin by using the mutant strains and a derivatization of cyclosporins by using *Sebekia benihana* KCTC 9173.

(Culture Conditions)

A Strain used for the production of cyclosporins is *Tolypocladium inflatum* ATCC 34921. The culture medium contained 50g of Glucose, 10 g of Peptone, 5 g of KH_2PO_4 2.5 g of KCl, and 4.4 mg of $ZnSO_4 \cdot 7H_2O$ per liter and the culture temperature was kept at 27 ℃ (see Appl. Microbol. Biotechnol., 1991; 34; 513 ~ 517).

Microorganisms used for the preparation of cyclosporin derivatives were *Sebekia Benihana* KCTC 9173 or *Pseudonocardia autotrophica* KCTC 9441. Culture medium contained 0.7% of glucose, 0.45% of yeast extract, 0.5% of malt extract, 1.0% of soluble starch, and 0.005% of calcium carbonate ($CaCO_3$), and the temperature was kept at 27 ℃ (J. Antibiotics, 1996; 49; 781 ~ 787).

To prepare an agar culture, agar was added in 20 g/l to the above medium. When using a fermentor, 4 ℓ fermentor with the medium was used, in which 4 day old preculture in an Erlenmeyer flask was used as an inoculum.

(Preparation of mutants and cyclosporins)

The agar medium with *Tolypocladium inflatum* ATCC 34921 grown was frozen in a quick freezing refrigerator. By scraping the surface of the agar medium mycelia were collected. The obtained mycelia were ground in a homogenizer and filtered through sterilized cotton to remove masses. The resultant filtrate was centrifuged at 3,000 ppm for 10 minutes to obtain spores. The collected spores were taken up in a 20% solution of glycerin and applied to an agar medium to determine a concentration of the spores.

For mutation by UV irradiation, about 10^4 C.F.U. (Colony Forming Unit) of the spores solution was applied to the agar medium and subjected to UV irradiation over 10 seconds to 1 minutes in a condition that around 10

colonies can be survived. The collected mutants were then measured for their productivity for cyclosporins to select the strains with high productivity.

After the selected strains were cultured for 10 days in the above culturing medium, cyclosporins in the culturing medium were extracted with ethyl acetate, concentrated and separated by means of HPLC.

Here, for HPLC separation C-18 column was used. The eluting solvent conditions were that 100% solvent A were flown for 2 minutes, concentration of solvent A were decreased to 60% by 4 minutes, and the concentration of solvent A were slowly decreased to 39% while eluting samples with concomitant increase of solvent B. Then, the condition returned to the original condition of 100% solvent A by 65 minutes. At this time, solvent A was 25% methanol aqueous solution and solvent B was 100% acetonitrile.

(Preparation of cyclosporin derivatives)

The strains used for preparing cyclosporin derivatives according to the present invention were *Sebekia benihana* KCTC 9173 or *Pseudonocardia autotrophica* KCTC9441. After 24 hour cultivation, cyclosporin B, C, D and G dissolved in methanol were added to the medium in 100 mg/ℓ , respectively and cultivated further for 72 hours.

After the cultivation, the whole mediums were extracted with ethyl acetate in the same amount to the medium. The organic solvent layer was separated and concentrated. The resulted sample was subjected to a high pressure liquid chromatography. FIG. 1 shows the high pressure liquid chromatography results of cyclosporin C and its derivative, [γ -hydroxy-N-methyl-L-leucine^4] cyclosporin C. FIG 2 shows a high speed liquid chromatography of purified [γ -hydroxy-N-methyl-L-leucine^4] cyclosporin C.

Here, for HPLC separation C-18 column was used. The eluting solvent conditions were that 100% solvent A were flown for 2 minutes, concentration of solvent A were decreased to 60% by 4 minutes, and the concentration of solvent A were slowly decreased to 39% while eluting samples with concomitant increase of solvent B. Then, the condition returned to the original condition of 100% solvent A by 65 minutes. At this time, solvent A was 25% methanol aqueous solution and solvent B was 100% acetonitrile.

(Confirmation of structure of cyclosporin derivatives)

A LCQ mass spectrometer (Finnigan, CA) using the ESI (electro-spray ionization) method was used in order to analyze the structure of collected cyclosporin derivatives. The tests were taken in a way of reciprocally comparing cyclosporin and cyclosporin derivatives.

In the Electro-Spray Ionization - Mass Spectrometer tests for confirming each molecular weight of cyclosporin B, C, D and G, a [M(cyclosporin + H) peak was measured in m/z. Cyclosporin derivatives showed {M(cyclosporin derivatives) + H} peaks at a m/z increased by 16 as compared to cyclosporin (for example, cyclosporin C derivatives showed at m/z 1234.9). Furthermore, the same tests were repeated with addition of sodium. From the above results, it could be presumed that cyclosporin derivatives have hydroxyl group added to cyclosporin molecules.

The CID (Collision Induced dissociation) method was used in order to confirm the position of the amino acid where the hydroxylation occurred among 11 amino acids of cyclosporin. After forming fragment ions by the collision induced dissociation method, the fragment ion pattern formed from cyclosporin and fragment ion pattern

formed from cyclosporin derivatives were comparatively analyzed. When referring to the fragment ion patterns, it was found that there was not any mass value changes in fragment ions of other amino acids, but the mass values of fragment ion peaks comprising No. 4 position amino acid were increased by 16. Therefore, it could be known that the transformation occurred at No. 4 position amino acid.

The nuclear magnetic resonance spectroscopy(ARX 600 MHz, Bruker, Germany) was additionally conducted in order to confirm that the added hydroxyl group was positioned at No. 4 amino acid, as was disclosed in the above test.

First, as comparing ^{13}C-Nuclear Magnetic Resonance spectrums of cyclosporin and cyclosporin derivatives, a new peak (δ 69.00 ppm) representing chemical shift of carbon comprising the added hydroxyl groups was observed.

In order to locate the carbon of this peak DEPT (distortionless enhancement by polarization transfer) tests were carried out. As the result, it was known that hydroxyl groups were attached to quaternary carbon, and this quaternization was made by adding hydroxyl group to γ -carbon position of No. 4 amino acids (hydroxylation). If quaternization had occurred to a α -carbon of No. 4 amino acid, the peak would have been shifted to down field near 90 ppm.

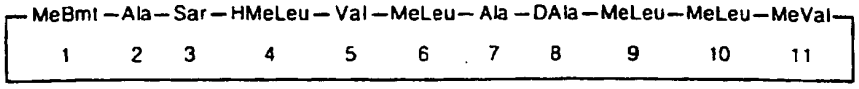
In addition, it can be known that a peak removed by microorganisms was near 25 ppm, and that one of 4 methine carbons, 4 N-methyl-L-leucine γ carbons in cyclosporin molecules, was removed.

Summing up, it could be known that hydroxyl group was added to No. 4 amino acid (N-methyl-L-leucine) from the result of mass spectrum method using electro-spray ionization and collision induced dissociation, and

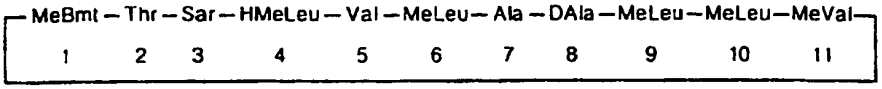
hydroxyl group was added to ɣ position carbon from the result of the nuclear magnetic resonance test.

From the above preparation and experiments, we have discovered that cyclosporin B, C, D and G are derivatives in which hydroxyl group was added to γ carbon of No. N-methyl-L-leucin in cyclosporin molecules, having the chemical formula as follows:

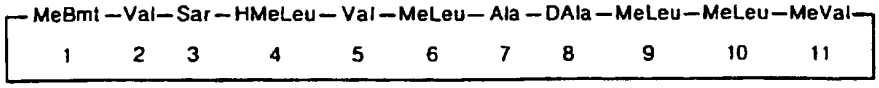
[Chemical Formula 1b]



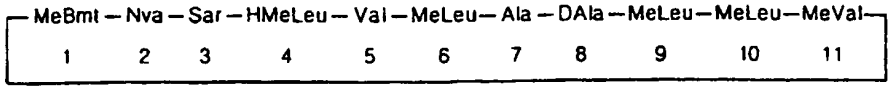
[Chemical Formula 1c]



[Chemical Formula 1d]



[Chemical Formula 1g]



where Ala is L-alanine; Thr is L-threonine; Val is L-valine; Nva is L-norvaline; MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine; Sar is Sarcosine; HMeLeu is γ-hydroxy-N-methyl-L-leucine; MeLeu is N-methyl-L-leucine; DAla is D-alanine; and MeVal is N-methyl-L-Valine. Furthermore, the amino acid form of the above cyclosporin A is L-configuration if there is not any mention.

TEST EXAMPLES

TEST EXAMPLE 1: test of hair growth promoting effects of cyclosporin derivatives of the invention

C57BL/6, female Mice (42 ~ 49 days old,) were used in the test of hair growth promoting effects.

First of all, several mice, after removing the backside hair using an electric shaver and weighing, were divided uniformly according to their weights. After one day of adaptation period, derivatives collected from HPLC of the above EXAMPLE 1 was applied on the area with hair removed in the amount of 100 μl (0.1% w/v) once a day per each individual for 30 days. The degrees of the hair growth were observed by naked eyes and determined by calculating the proportion of area where new hairs were grown compared to the area in which hairs originally were removed.

As seen in Table 1, the remarkable hair growth promoting effects by cyclosporin derivatives were shown compared to the control group in which only vehicle were applied, and their activities was comparable to cyclosporin A. The difference of before and after the transformation was very much inappreciable.

Upon observing the back conditions of mice during 30 days of test procedure, the appreciable skin irritations were not found from the control group and all treated groups.

Table 1

	Proportion of area (%)
Vehicle	32
Cyclosporin A	87
Cyclosporin B derivative	80
Cyclosporin C derivative	88
Cyclosporin D derivative	78
Cyclosporin G derivative	86

TEST EXAMPLE 2: immunosuppression tests of cyclosporin derivatives

According to the MLR method (Mixed Allogenic Mixed Lymphocyte Reaction method) by mixing spleen cells of two different species of mouse (J. Antibiotics, 1994; 47; 208 ~ 215) was used in the immunosuppression comparison test was carried out.

After mixing the equivalent numbers of BALB/c mouse spleen cells as reacting cells and mitomycin treated C57BL/6 mouse spleen cells as stimulating cells, the mixture was treated with various cyclosporins. And then, it was cultured in RPMI medium containing mercaptoethanol and 10% fetal bovine serum for 4 days. ^{3}H-thymidine was added to the solution and cultured for further 4 hours. After culturing, IC_{50}($\mu g/m\ell$) of each materials was calculated by liquid scintillation countering the amount of thymidine influxed into cells.

As the results of that, IC_{50}($\mu g/m\ell$) of cyclosporin A showed 0.035 while IC_{50}($\mu g/m\ell$) of cyclosporin B, C, D and G derivatives showed 6.3, 6.6, 5.9 and 9.6, indicating over 100 times decrease of immunosuppression. This was the similar level to the literature (J. Antibiotics,

1996, 49, 781 ~ 787, and J. Virol., 1995; 69; 2451 ~ 2461).

That is, it was found that a group of cyclosporin derivatives according to the present invention in which hydroxyl group is added to the γ -carbon position of No. 4 N-methyl-L-leucine in cyclosporin by the microorganism metabolism procedure had much lower degree of immunosuppression than that cyclosporin A and also, their own non-transformed derivatives while maintained superior hair growth effects.

FORMULATIONS

FORMULATION 1-1: preparation of a hair revitalization tonic containing cyclosporin C derivatives

A hair- revitalization tonic was prepared in 3 types of preparation forms represented in the following Table 2 by mixing, agitating, and completely dissolving each raw material. As comparing the trichogenous effects of the hair growing tonic of Preparation Form 1 in Table 2 with a tonic containing cyclosporin A (0.1 %) according to the above Test Example 1, it was found that the efficiency of the present tonic was similar to that comprising cyclosporin A.

Table 2

Ingredient (wt%)	Preparation Form		
	Form 1	Form 2	Form 3
Ethanol	40.0	40.0	40.0
Cyclosporin C derivative	0.1	1.0	8.0
Tocopherol acetic acid	0.1	0.1	0.1
Salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Tween 20	0.5	0.5	0.5
Perfume	Approp. amount	Approp. amount	Approp. amount
Colorant	Approp. amount	Approp. amount	Approp. amount
Water	Balance	Balance	Balance

FORMULATION 1-2: preparation of a hair revitalization tonic containing cyclosporin G derivatives

A hair- revitalization tonic was prepared in 3 types of preparation forms represented in the following Table 3 by mixing, agitating, and completely dissolving each raw material. As comparing the trichogenous effects of the hair revitalization tonic of Preparation Form 1 in Table 3 with a tonic containing cyclosporin A (0.1 %) according to the above Test Example 1, it was found that the efficiency of the present tonic was similar to that comprising cyclosporin A.

Table 3

Ingredient (wt%)	Preparation Form		
	Form 1	Form 2	Form 3
Ethanol	40.0	40.0	40.0
Cyclosporin G derivative	0.1	1.0	8.0
Tocopherol acetic acid	0.1	0.1	0.1
Salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Tween 20	0.5	0.5	0.5
Perfume	Approp. amount	Approp. amount	Approp. amount
Colorant	Approp. amount	Approp. amount	Approp. amount
Water	Balance	Balance	Balance

Formulation 2-1: preparation of a hair cream containing cyclosporin C derivatives

Oil soluble raw materials and water soluble raw materials among raw materials in 3 types of preparation forms represented in the following Table 4, were separately mixed while heating to 80 ℃ to completely dissolve them. The resulted mixtures of the prepared oil soluble raw materials and water soluble raw materials of 80 ℃ were mixed together and emulsified. After completing the emulsification and cooling to the room temperature, hair cream was prepared by adding and mixing perfume and colorant. The amount of water added was calculated so that total composition contents of each preparation form could be adjusted to 100 wt%. As comparing the trichogenous effects of the hair tonic of Preparation Form 1 in Table 4 with a tonic containing cyclosporin A (0.1 %) according to the above Test Example 1, it was found that the efficiency of the present tonic was similar to that comprising cyclosporin A.

Table 4

Ingredient (wt%)		Preparation Form		
		Form 1	Form 2	Form 3
Oil soluble raw materials	Paraffin	5.0	5.0	5.0
	Setostearylalcohol	5.5	5.5	5.5
	Petrolatum	5.5	5.5	5.5
	Glycerine -monostearate	3.0	3.0	3.0
	Polyoxyethylene octyldodecylether	3.0	3.0	3.0
	Propylparaben	0.3	0.3	0.3
	Cyclosporin C derivative	0.1	1.0	8.0
Water soluble raw materials	Glycerin	7.0	7.0	7.0
	Dipropylene glycol	20.0	20.0	20.0
	Polyethylene glycol	5.0	5.0	5.0
	Water	45.6	44.7	37.7
Perfume		Approp. amount	Approp. amount	Approp. amount
Colorant		Approp. amount	Approp. amount	Approp. amount

Formulation 2-2: preparation of a hair cream containing cyclosporin G derivatives

Oil soluble raw materials and water soluble raw materials among raw materials in 3 types of preparation forms represented in the following Table 5, were separately mixed while heating to 80 ℃ to completely dissolve them. The resulted mixtures of the prepared oil soluble raw materials and water soluble raw materials of 80 ℃ were mixed together and emulsified. After completing the emulsification and cooling to the room temperature, a hair cream was prepared by adding and

mixing perfume and colorant. The amount of water added was calculated so that total composition contents of each preparation form could be adjusted to 100 wt%. As comparing the trichogenous effects of the hair tonic of Preparation Form 1 in Table 5 with a tonic containing cyclosporin A (0.1 %) according to the above Test Example 1, it was found that the efficiency of the present tonic was similar to that comprising cyclosporin A.

Table 5

Ingredient (wt%)		Preparation Form		
		Form 1	Form 2	Form 3
Oil soluble raw materials	Paraffin	5.0	5.0	5.0
	Setostearylalcohol	5.5	5.5	5.5
	Petrolatum	5.5	5.5	5.5
	Glycerine-monostearate	3.0	3.0	3.0
	Polyoxyethylene octyldodecylether	3.0	3.0	3.0
	Propylparaben	0.3	0.3	0.3
	Cyclosporin G Derivative	0.1	1.0	8.0
Water soluble raw materials	Glycerin	7.0	7.0	7.0
	Dipropylene glycol	20.0	20.0	20.0
	Polyethylene glycol	5.0	5.0	5.0
	Water	45.6	44.7	37.7
Perfume		Approp. amount	Approp. amount	Approp. amount
Colorant		Approp. amount	Approp. amount	Approp. amount

FORMULATION 3-1: preparation of a shampoo containing cyclosporin C derivatives

Raw materials except perfume, colorant, and water in 3 types of preparation forms represented in the following Table 6 were mixed, until they were completely dissolved by heating while agitating. After cooling the mixture to the room temperature and adding perfume and colorant to it, finally water was added so that total composition content could be adjusted to 100 wt% to obtain a shampoo.

Table 6

Ingredient (wt%)	Preparation Form		
	Form 1	Form 2	Form 3
Sodium POE laurylsulfuric acid (30 wt% aqueous solution)	40.0	40.0	40.0
Coconut oil fatty acid Diethanolamide	3.0	3.0	3.0
1,2-propylene glycol	2.0	2.0	2.0
Methyl paraoxybenzoic acid	0.2	0.2	0.2
Ethanol	2.0	2.0	2.0
Cyclosporin C derivative	1.0	3.0	10.0
Salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Perfume	Approp. amount	Approp. amount	Approp. amount
Colorant	Approp. amount	Approp. amount	Approp. amount
Water	Balance	Balance	Balance

FORMULATION 3-2: preparation of a shampoo containing cyclosporin G derivative

Raw materials except perfume, colorant, and water in 3 types of preparation forms represented in the following Table 7 were mixed, until they were completely dissolved by heating while agitating. After cooling the mixture to the room temperature and adding perfume and colorant to it, finally water was added so that total composition content could be adjusted to 100 wt% to obtain a shampoo.

Table 7

Ingredient (wt%)	Preparation Form		
	Form 1	Form 2	Form 3
Sodium POE laurylsulfuric acid (30 wt% aqueous solution)	40.0	40.0	40.0
Coconut oil fatty acid Diethanolamide	3.0	3.0	3.0
1,2-propylene glycol	2.0	2.0	2.0
Methyl paraoxybenzoic acid	0.2	0.2	0.2
Ethanol	2.0	2.0	2.0
Cyclosporin G derivative	1.0	3.0	10.0
Salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Perfume	Approp. amount	Approp. amount	Approp. amount
Colorant	Approp. amount	Approp. amount	Approp. amount
Water	Balance	Balance	Balance

FORMULATION 4-1: preparation of hair conditioner containing cyclosporin C derivative

Oil soluble materials and water soluble materials among raw materials were separately mixed in 3 types of preparation forms represented in the following Table 8 and completely dissolved by heating up to 80 ℃. The prepared mixtures of oil soluble raw materials and water soluble raw materials of 80 ℃ were mixed together and emulsified. After the emulsification and cooling to the room temperature, a hair conditioner was prepared by adding and mixing perfume and colorant.

The amount of water was added so that total composition content preparation could be adjusted to 100 wt%.

Table 8

Ingredient (wt%)		Preparation Form		
		Form 1	Form 2	Form 3
Oil soluble raw materials	Cetanol	3.0	3.0	3.0
	Self-emulsion type Glycerol monostearate	2.0	2.0	2.0
	Squalene	10.0	10.0	10.0
	Cyclosporin C Derivative	1.0	5.0	10.0
Water soluble raw materials	Propylene glycol	2.0	2.0	2.0
	Stearyldimethyl Benzylammonium chloride (25 wt% aqueous solution)	8.0	8.0	8.0
	Methyl paraoxybenzoic acid	0.2	0.2	0.2
	Salicylic acid	0.3	0.3	0.3
	L-menthol	0.3	0.3	0.3
	Water	73.2	69.2	64.2
Perfume		Approp. amount	Approp. amount	Approp. amount
Colorant		Approp. amount	Approp. amount	Approp. amount

FORMULATION 4-2: preparation of hair conditioner containing cyclosporin G derivative

Oil soluble materials and water soluble materials among raw materials were separately mixed in 3 types of preparation forms represented in the following Table 9 and completely dissolved by heating up to 80 ℃. The prepared mixtures of oil soluble raw materials and water soluble raw materials of 80 ℃ were mixed together and emulsified. After the emulsification and cooling to the

room temperature, a hair conditioner was prepared by adding and mixing perfume and colorant.

The amount of water was added so that total composition content preparation could be adjusted to 100 wt%.

Table 9

Ingredient (wt%)		Preparation Form		
		Form 1	Form 2	Form 3
Oil soluble raw materials	Cetanol	3.0	3.0	3.0
	Self-emulsion type Glycerol monostearate	2.0	2.0	2.0
	Squalene	10.0	10.0	10.0
	Cyclosporin G Derivative	1.0	5.0	10.0
Water soluble raw materials	Propylene glycol	2.0	2.0	2.0
	Stearyldimethyl Benzylammonium chloride (25 wt% aqueous solution)	8.0	8.0	8.0
	Methyl paraoxybenzoic acid	0.2	0.2	0.2
	Salicylic acid	0.3	0.3	0.3
	L-menthol	0.3	0.3	0.3
	Water	73.2	69.2	64.2
Perfume		Approp. amount	Approp. amount	Approp. amount
Colorant		Approp. amount	Approp. amount	Approp. amount

Industrial Applicability

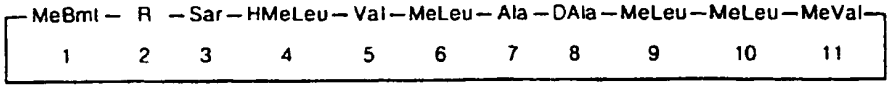
A hair growth promoter comprising an active ingredient of cyclosporin B, C, D, and G derivatives according to the present invention has a much lower degree of immunosuppression compared to cyclosporin A while maintains the excellent hair growth. Therefore, it is expected that the present invention has better trichogenous effect than the conventional agents based on either minoxidil or finasteride.

Although the preferred embodiments of the invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

WHAT IS CLAIMED IS:

1. A hair growth promoter comprising an active ingredient of nonimmunosuppresive cyclosporin derivatives represented as the following Chemical Formula 1 in which hydroxyl group is added to γ carbon position of No. 4 N-methyl-L-leucine of the cyclosporin molecules by the microorganism metabolism procedure:

[Chemical Formula 1]



where R is Ala, Thr, Val or Nva, in which Ala is L-alanine, Thr is L-threonine, Val is L-valine, Nva is L-norvaline; MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine; Sar is Sarcosine; HMeLeu is γ-hydroxy-N-methyl-L-leucine; MeLeu is N-methyl-L-leucine; DAla is D-alanine; and MeVal is N-methyl-L-Valine.

2. The hair growth promoter in accordance with claim 1, wherein R is L-alanine.

3. The hair growth promoter in accordance with claim 1, wherein R is L-threonine.

4. The hair growth promoter in accordance with claim 1, wherein R is L-valine.

5. The hair growth promoter in accordance with claim 1, wherein R is L-norvaline.

6. The hair growth promoter in accordance with claim 1, which is prepared in one or more forms selected

from the group consisting of liquid formulation, spray, gel, paste, emulsion, cream, conditioner, and shampoo.

FIGURE

FIG. 1

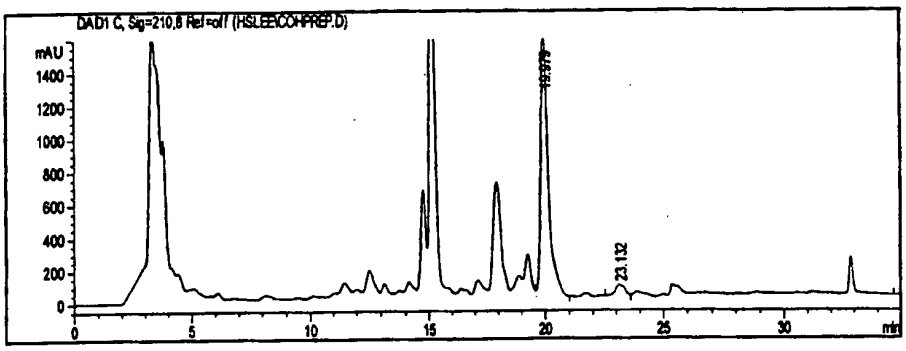
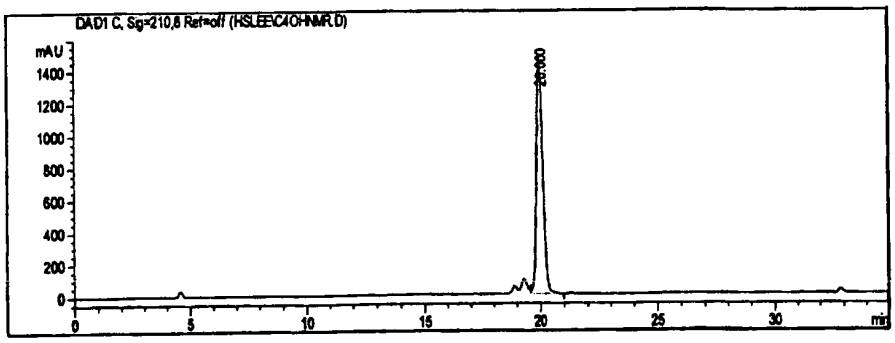


FIG. 2



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR00/01301

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 7/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)

IPC7 A61K 7/06

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Korean Patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)

CAPLUS (STN), NPS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MAURER et al. , ' Hair growth modulation by topical immunophilin ligands: induction of anagen, inhibition of massive catagen development, and relative protection from chemotherapy-induced alopecia' , Am. J. Pathol., American Society for Investigative Pathology, US, 1997, 150(4), p 1433-1441	1 - 6
A	YAMAMOTO et al., ' Hair growth-stimulating effects of cyclosporin A and FK506, potent immunosuppressants, J. Dermatol. Sci., 1994, 7(Suppl.), S47-S54	1 - 6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
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"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 FEBRUARY 2001 (15.02.2001)	16 FEBRUARY 2001 (16.02.2001)

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